American Journal of Pathology, Vol. 139, No. 6, December 1991

Rapid Communication

Early Viral Replication in the Brain of SIV–infected Rhesus Monkeys

Lisa Chakrabarti,* Maryse Hurtrel,† Marie-Annick Maire,* Rosemay Vazeux,* Dominique Dormont,‡ Luc Montagnier,* and Bruno Hurtrel*

From the Unité d'Oncologie Virale, Institut Pasteur, Paris, l'Ecole Nationale Vétérinaire,† Nantes, and Centre de Recherche du Service de Santés des Armées,† Fontenay aux Roses, France*

To investigate the mechanism of simian immunodeficiency virus (SIV) entry into the central nervous system (CNS) and the initial events leading to neuropathogenesis, SIV replication was studied by* in situ *hybridization in the CNS of 5 Rhesus macaques at 7 days, 1, 2, and 3 months after SIV intravenous inoculation. CNS infection was found to be a frequent and early event, as SIV was detected in the CNS of all the animals studied and as early as 7 days postinoculation. At the earliest stage, the infection localized mainly to perivascular cells. Using combined immunohistochemistry and* in situ *hybridization, infected cells were shown to express the CD68 marker, suggesting that infected mononuclear phagocytes crossing the blood-brain barrier represent the main source of virus in the CNS. Early viral replication coincided with neuropathologic changes, consisting in gliosis, perivascular infiltrates and rare glial nodules. Immunophenotyping of brain tissue showed that increased macrophage infiltration, microglial reactivity and MHC class II induction occurred within the first week of infection, indicating a possible immunopathologic mechanism in early CNS pathogenesis. (Am J Pathol 1991, 139:1273–1280)

Neurologic impairment is one of the most severe complications associated with human immunodeficiency virus (HIV) infection. A progressive dementia afflicts many patients with the acquired immunodeficiency syndrome (AIDS)[1,2] and at autopsy, more than 70–80% present abnormalities of the nervous system.[3] Characteristic pathologic findings consist of microglial foci, multinucleated giant cells, and diffuse damage to the white matter.[33] The possible mechanisms of HIV neuropathogenesis remain speculative and in particular, little is known concerning how HIV initially gains access to the central nervous system (CNS). The occasional presence of HIV-specific antibodies[4,5] or HIV virions[6] in the cerebrospinal fluid (CSF) of patients in the asymptomatic phase of the disease indicates that CNS infection by HIV may occur early. Cases of acute encephalopathy coincident with seroconversion for HIV have been reported[7,8] and a small but significant number of patients exhibit a neurologic disorder as an early manifestation of HIV infection, before the occurrence of secondary opportunistic infections.[9] Electroencephalographic abnormalities were found to occur in 30–40% of asymptomatic seropositive men, indicating that subclinical neurologic impairment may be a common feature early in the course of HIV infection.[10]

Direct evidence of HIV replication in the CNS has been obtained for the latter stages of infection, as viral nucleic acids and proteins were conclusively demonstrated in cells of the monocyte/macrophage lineage within the brains of AIDS patients.[11–13] High levels of unintegrated HIV-1 DNA in brain tissue were found to correlate with the occurrence of HIV-1 encephalitis.[14] A similar correlation was found between the extent of HIV expression and the severity of the lesions in spinal cord tissue.[15] These studies suggest a direct involvement of HIV in the generation of nervous system dysfunction.

Due to the inherent difficulty in obtaining human specimen early after HIV infection, a relevant animal model appears indispensable to better understand the mode of entry of HIV in the CNS and the early phase of the neuropathology. Infection of rhesus macaques with simian immunodeficiency virus (SIV) provides such a model, as SIV is a retrovirus closely related to HIV in its pathogenicity[16] and genetic structure.[17–19] SIV induces an encephalitis in macaques similar to the neurologic lesions seen in patients with AIDS[20] and with a comparable frequency.[21]

Supported by the Comité National d'Action contre le SIDA and Agence Nationale de Recherches sur le SIDA (France).

Accepted for publication October 10, 1991.

Address reprint requests to Dr. Lisa Chakrabarti, Unité d'Oncologie Virale, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris, Cedex 15, France.

In the present study, we used *in situ* hybridization and immunohistochemistry to examine SIV replication in brain tissue of macaques at different times after intravenous inoculation. SIV replication was detected in perivascular brain macrophages during the initial viremic phase, demonstrating that infection of the CNS is a remarkably early event.

Materials and Methods

Animals and Specimens

Six female Rhesus monkeys (*Macaca mulatta*) aged between 3 and 5 years were included in the study. Five of these animals were inoculated intravenously with 450 tissue culture infectious doses of the SIVmac 251 isolate. Before inoculation, the animals were demonstrated to be seronegative for STLV-1, SRV-1 (type D retrovirus), Herpes B virus and SIVmac. Viremia was detected by cocultivation of monkey peripheral blood mononuclear cells (PBMC) with uninfected human PBMC and by measurement of p25 gag antigen in monkey sera, using an HIV-1 antigen capture Elisa test (Abbott). Antibody response to SIV was monitored by radioimmunoprecipitation. The T4/T8 ratio was determined by flow cytometry, after labelling with the OKT4 (Ortho-mune) and Leu2a (Beckton Dickinson) antibodies.

The animals were sacrificed at 7 days and 1, 2, and 3 months postinoculation. Classical histopathologic examination was performed on all the organs collected and a systematic study was carried out on the CNS samples. The cerebrum, cerebellum, and brain stem were subdivided. Part of the samples were frozen for further analysis by immunostaining and *in situ* hybridization. The other part was fixed in formalin and subjected to histopathologic examination. Organs obtained from the control uninfected rhesus macaque were processed similarly.

Immunohistochemistry

Immunohistochemical staining was performed in three steps, using horseradish peroxidase-coupled secondary and tertiary antibodies, with diaminobenzidine as the chromogen. Monoclonal antibodies Leu 5b (anti-CD2, Becton Dickinson), Leu 2a (anti-CD8, Becton Dickinson), OKT4 (anti-CD4, Ortho-mune), KiM7 (anti-CD68, Behring), L243 (anti-HLA DR, ATCC hybridoma HB55, ascite provided by G. Sterkers) were used to characterize inflammatory infiltrates in infected macaque brains. Astrocytes were detected with a monoclonal antibody directed against glial fibrillary acidic protein (GFAP, Amersham) and neurons by a monoclonal antibody against neurofilament (Dakopatts). SIV capsid antigen was detected with a mixture of anti-p25 monoclonal antibodies (anti-HIV-1 p25 15-42 provided by B. S. Parekh, Biorad and anti-HIV-2 208.3 from Hybridolab Pasteur). The presence of CNS opportunistic pathogens was tested with antibodies to toxoplasma (1E10, IV47 and G119/2, Biosoft), CMV (E13, Biosoft) (A95/30, Michelson), SV40 (agT, Oncogene) and herpes viruses (HSV1, HSV2 and CHA437, Biosoft).

In Situ *Hybridization*

Hybridization techniques were based on published procedures.[22] RNA probes were derived from the transcription vector Bluescript (from Vector Cloning Systems) in which a fragment of the SIVmac142 plasmid clone[17] spanning the *nef* gene (nucleotides 8718 to 8234) was inserted. The antisense probe aPnef used to detect SIV RNA was generated from the T7 promoter by *in vitro* transcription of 1 μg of plasmid template with 50 units of T7 RNA polymerase in the presence of 50 μCi of ^{35}S UTP and ^{35}S ATP. Specific activity ranged between 1.10^8 and 5.10^8 cpm/mg of input DNA.

Controls included 1) hybridization of SIV infected and uninfected lymphocytes, 2) hybridization of brain tissue from an uninfected monkey, 3) hybridization with an RNA probe unrelated to SIV.

Combined Immunohistochemistry and In Situ *Hybridization*

Procedures were modified from those described by Brahic et al.[23] Tissue sections were incubated in 4% paraformaldehyde for 20 mn, rinsed in phosphate-buffered saline, and then processed for immunohistologic staining. For the saturation step, normal serum was replaced by 0.2% tween and 1% RNAse free BSA to minimize RNAse contamination. The same tissue sections were then refixed in 4% paraformaldehyde for 10 mn and processed for *in situ* hybridization as previously described.[22]

Results

Neuropathologic Findings

SIV-inoculated rhesus macaques presented a spectrum of brain changes at the early stages of infection (Table 1). The earliest pathologic changes observed were a disseminated gliosis, i.e., increase in non-neuronal cells, and rare glial nodules (Figure 1A) in the temporal cortex of animal 40481 at 7 days postinoculation (p.i.). Gliosis was a consistent finding in the brain of all infected ani-

Table 1. *Neuropathologic Findings and Virus Detection in the CNS of Macaques Inoculated Intravenously with SIV*

	Animal identity (time postinoculation, days)					
	40481 (7)	40498 (30)	40499 (65)	40484 (78)	40488 (98)	42895 (n.i.)
Lymph node hyperplasia	−	+	−	+	++	−
Anti SIV antibodies	ND	+	−	+	+	−
Viremia (p25 ng/ml)	1,03	<0.02	14.4	<0.02	<0.02	<0.02
Neuropathology						
Gliosis	+	++	++	++	++	−
Glial nodules	+	+	++	−	+	−
Perivascular infiltrates	−	+	++	+	+	−
White matter pallor	−	−	++	−	++	−
Giant cells	−	−	++	−	−	−
Meningitis	−	−	++	−	−	−
Spongiosis	−	−	−	++	−	−
Virus detection in CNS						
Immunohistochemistry						
p25 antigen	+	+	+++	+	−	−
In Situ hybridization						
Frontal lobe	+	+	++	−	−	−
Central region: basal ganglia	−	−	++	−	−	−
Central region: white matter	++	+	+++	+	+	−
Temporal lobe	+	+	+++	+	+	−
mean number of infected cells (per 2 cm^2 in central region)	8	3	200	<1	2	0

Findings in infected animals were compared with those in an uninfected animal (n.i., noninfected). Histopathologic examination was performed on 7 different regions of formalin fixed brain tissue. Lesions are scored as follows: − absent; + moderate; ++− severe. Immunohistochemistry and *in situ* hybridization were performed on three different regions of frozen brain tissue. For *in situ* hybridization, viral RNA and DNA were detected with an SIV-specific nef gene probe. The degree of infection is scored as follows: +, 1–5 infected cells; ++, 5–20 infected cells; +++, >20 infected cells per 2 cm^2.

mals and was not found in brain sections of an uninfected control animal. Hallmarks of inflammation, including numerous microglial nodules and mononuclear cell infiltrates surrounding blood vessels and leptomeninges were observed for three of the animals, and was detected as early as 30 days p.i. for animal 40498. Severe encephalitis characterized by massive mononuclear infiltrates, white matter pallor, and multinucleated giant cells was observed for animal 40499. This animal had not mounted an anti-SIV antibody response at 65 days p.i., indicating immunologic dysfunction. At 78 days p.i., animal 40484 had few mononuclear infiltrates in the brain but exhibited randomly dispersed vacuoles in the white matter, a finding similar to the spongiform encephalopathy occasionally described in humans.[24] At 3 months p.i., animal 40498 presented a mild encephalitis with glial nodules and demyelinated areas. Thus, neuropathologic changes were observed within the first month of infection (animals 40481 and 40498) and were more severe at the later stages of infection (animals 40499, 40484, and 40488).

Virus Detection

We sought to determine whether the early neuropathologic changes observed were associated with active viral replication in the CNS. Using monoclonal antibodies directed against HIV p25 core protein, the presence of SIV antigens could be demonstrated in the brain of four of five infected animals (Table 1). In the case of severe encephalitis (40499), numerous infected cells were observed mostly at the sites of microglial nodules and near the leptomeninges. Immunohistochemic staining for opportunistic CNS pathogens, including toxoplasma, SV40, and herpes viruses was negative in brain tissue of all the animals studied.

To detect and quantitate rare isolated SIV infected cells, we used the *in situ* hybridization technique. For each animal, tissue sections of the frontal lobe, basal ganglia region and temporal lobe were screened with a SIV-specific riboprobe. A nef gene probe was chosen because all types of SIV RNAs, genomic as well as singly or doubly spliced, hybridize to this region of the genome. Using the *in situ* hybridization technique, infected cells were detected in the brain of all the intravenously inoculated macaques and not in the control animal (Table 1). Viral replication occurred in the brain of animal 40481 as early as 7 days p.i., and 70% of the infected cells detected were localized in a perimeningeal or perivascular area. The perivascular infected cells taken into account were observed in the brain parenchyma in the vicinity of blood vessels but were not enclosed within the vascular wall (Figure 1C), thus representing cells that had actually crossed the blood-brain barrier. Animal 40498 presented another case of early viral replication in the CNS, scat-

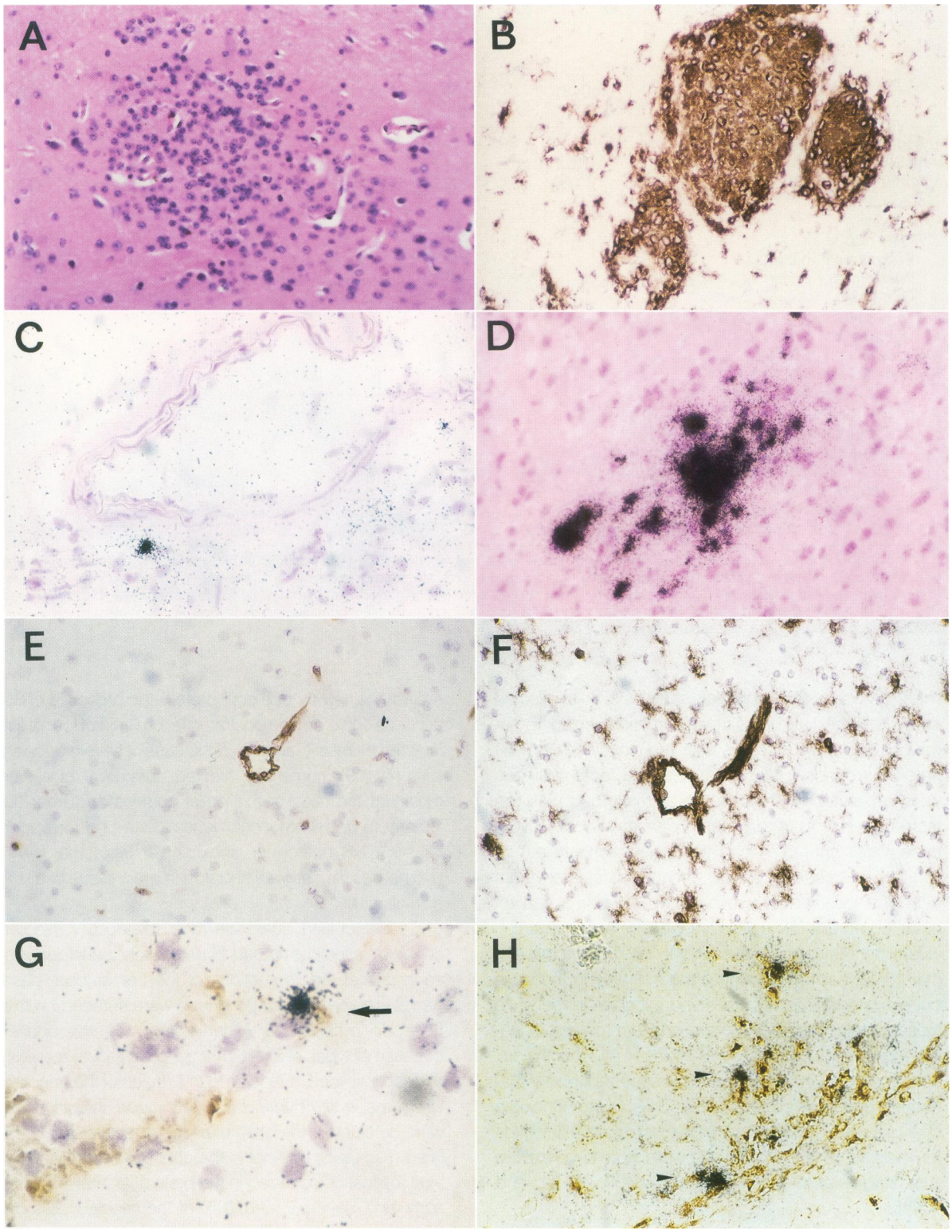

Figure 1. *Histopathology,* in situ *hybridization, and immunohistochemical staining of brain tissue from SIV infected macaques. For each photograph, animal identity and magnification are indicated in parentheses.* **A**: *Glial nodule observed in the temporal lobe 7 days postinoculation, H & E staining (40481; ×125).* **B**: *KiM7 immunohistochemical staining of nodule macrophages and scattered microglial cells (40499, ×125).* **C, D**: *SIV detection by* in situ *hybridization with a* ^{35}S *labelled probe.* **C**: *Perivascular infected cell detected in the CNS 7 days p.i. (40481; ×500).* **D**: *Massive SIV replication in a glial nodule, in a case of severe encephalopathy (40499, ×500).* **E, F**: *Immunohistochemical detection of MHC class II molecules in the brain, using anti-HLA DR staining.* **E**: *MHC class II expression restricted to neuroendothelial cells in the brain of a control animal (42895, ×500).* **F**: *Induction of MHC class II expression in microglial cells of an infected animal early after SIV inoculation (40481, ×500).* **G, H**: *Identification of infected cells by combined* in situ *hybridization and immunohistochemistry. Brain sections were subsequently labeled with KiM7 monoclonal antibody and hybridized with a SIV specific probe. KiM7+ infected cells are detected by the superposition of brown color and black silver grains.* **G**: *Perivascular infected brain macrophage (arrow) detected 7 days p.i. (40481, ×1500).* **H**: *Infected macrophages (arrows) in a perimeningeal inflammatory infiltrate (40499; ×310).*

tered infected cells being detected in the white matter and perivascular area at 30 days p.i. Massive SIV replication took place in the brain of animal 40499 presenting a severe encephalitis (Figure 1D). A correspondingly high viral load was found in the blood of the same animal, as measured by p25 antigenemia (data not shown). In this limited experimental series, no positive correlation was found between the level of SIV replication and the severity of the neuropathologic changes, as the two animals at the later stages of infection (40484 and 40488) presented marked encephalopathy with spongiosis or white matter lesions, whereas minimal number of SIV infected cells were detected in the brain (Table 1). Thus, infection of the brain by SIV was shown to be a frequent and early event, though viral replication appeared to be maintained at restricted levels in most of the animals studied.

Immunophenotyping of Infected Brain Tissue

To further characterize the neuropathologic changes induced by SIV, we analyzed by immunohistochemistry the phenotype of infiltrated cells and the modifications of cell surface marker expression of resident cells in infected brain tissue. Marked changes were found in cells of the mononuclear phagocytic system. In an uninfected macaque brain, two populations of cells expressing the CD68 marker (KiM7+) were detected: large perivascular macrophages and numerous process bearing microglial cells disseminated in the brain parenchyma. The perivascular macrophage population was markedly increased at the early stages of infection and in the case of severe encephalitis (Table 2). Glial nodules, characteristic of pathologic brain, were mostly macrophage derived (Figure 1B). Reactive microglia presenting increased CD68 expression and more visible cytoplasmic processes was a consistent finding in the brain of all the infected animals, and was more pronounced at the early stages of infection (Table 2). Conforting these findings, an induction of the major histocompatibility complex (MHC) class II molecules was observed in infected brain. MHC class II expression was restricted to endothelial cells in the control (Figure 1E), whereas it was found in both endothelial and microglial cells in infected brain (Figure 1B). Additional changes included presence of T cells (Leu5b+), which were not found in normal brain. T cells represented a minor part of the perivascular infiltrates and were mostly of the T8 phenotype (Table 2). Staining with anti-GFAP revealed two cases of astrocytosis, with a marked increase in the number of astrocytes in the grey matter for animal 40488 at 3 months p.i. Taken together, immunohistochemical analysis indicated activation of whole subsets of the non-neuronal brain cell population even though the number of infected cells was low.

Characterization of Infected Cells

We used combined immunohistochemistry and *in situ* hybridization techniques to analyze the phenotype of infected cells. SIV RNA containing cells were found to express the CD68 marker (KiM7+). Infected cells belonged mostly to the perivascular macrophages population (Figure 1G, 1H) but were also found in macrophages constituting glial nodules and in parenchymal microglial cells. Infected cells in the brain were not found positive for the CD4 marker ($OKT4^-$), though CD4 infected cells were detected in lymph nodes of the same animals used as positive controls. These results indicated that the major source of the virus in the brain was derived from macrophages expressing undetectable levels of CD4 and not from infiltrating T4 lymphocytes expressing high levels of CD4. Astrocytes stained for GFAP and neurons stained for neurofilament were not found to contain SIV RNA. The phenotype of the infected cells associated with their perivascular localization in the early stages of infection support the notion that SIV initially gains access to the CNS within infected monocytes/macrophages crossing the blood-brain barrier.

Table 2. *Immunophenotyping of Brain Tissue in Macaques Inoculated Intravenously with SIV*

	Animal identity (time postinoculation days)					
	40481 (7)	40498 (30)	40499 (65)	40484 (78)	40488 (98)	42895 (n.i.)
Astrocytosis (GFAP)	+	−	−	−	++	−
Perivascular macrophages (KiM7)	+	+	++	−	−	−
Microglial reactivity (KiM7)	++	++	++	+	+	−
MHC class II induction (HLA DR)	++	++	++	−	++	−
Infiltrated T lymphocytes (Leu5b)	+	++	++	+	+	−
T8 lymphocytes (Leu2a)	15	>100	>100	7	7	1
T4 lymphocytes (OKT4)	4	18	8	0	3	0

The monoclonal antibodies used are indicated in parentheses. Immunohistochemistry was performed on frontal lobe and basal ganglia sections of frozen brain tissue. On the two bottom lines are indicated the mean number of T8 and T4 cells counted per 2 cm^2 section. (n.i., noninfected). Intensity of staining: −, same as uninfected control; +, moderate increase; ++, marked increase.

Discussion

We addressed the question of when and how is the brain infected in the course of SIV infection. Several mechanisms for entry of lentiviruses in the CNS have been described in the literature, favoring either infiltration of mobile cells carrying the virus in a repressed state (the Trojan horse hypothesis)[25] or direct infection of brain parenchyma by cellfree virus. It is generally believed that HIV is carried across the blood-brain barrier within infected mononuclear phagocytes.[12,13] CSF studies[5,6] and reported cases of early neurologic impairment[7–9] suggest that the CNS could be affected early in the course of HIV1 infection. However, in light of the frequent association of opportunistic pathogens and HIV in the brain of patients with nervous dysfunction, it has been proposed that HIV could secondarily gain access to the CNS.[26] Opportunistic infections in the CNS could trigger an inflammatory process, leading to the recruitment of HIV infected macrophages in the CNS of immunocompromised patients. Alternate mechanisms propose that free extracellular virus could cross the blood-brain barrier after replication in vascular endothelium cells[28] or could infect the CSF via replication in choroid plexus cells and subsequently spread to the brain parenchyma.[29]

We present direct evidence, in the SIV model, that infection of the CNS is an early event. SIV replication could be detected in macaque brain as early as 1 week p.i. and was also found in the brain of all the animals studied at 1, 2, and 3 months p.i. SIV replication in the brain coincided with early neuropathologic changes consisting in the presence of gliosis, glial nodules, and perivascular infiltrates. Abnormalities in the brain cell populations, including activation of microglial cells expressing higher levels of the CD68 and MHC class II molecules, increase in perivascular macrophages and presence of T lymphocytes in the CNS occurred within the first week of infection. As common CNS opportunistic pathogens such as toxoplasma, SV40, and herpes viruses were not detected in the animals studied, these findings support the notion that SIV plays a primary role in the generation of encephalitis, though additional opportunistic infections can certainly contribute to the neuropathology later in the course of the disease. The results we report in the simian model, in conjunction with indirect evidences obtained from HIV-infected humans, strongly suggest an early involvement of the CNS in the course of HIV infection. Considering the early presence of the virus in the CNS, it is likely that HIV replication in the CNS is initiated early and later reactivated rather than initiated by immunosuppression and additional infections. A parallel can be drawn between the primary and late phases of HIV (or SIV) infection. Both are characterized by high viremia, high circulating levels of activation markers such as neopterin, and by an inefficient antiviral immune response.[30] Thus, the same physiologic parameters may induce viral expression in the CNS during both phases of the disease.

A high level of SIV replication was observed in the brain of 40499 that presented a lack of antiviral immunity, indicated by absence of anti-SIV antibody response and by lymph node aplasia. To a lesser extent, a rather high viral load was observed in the brain of the animal in the initial viremic phase (40481), before the antiviral response could develop. Conversely, animals 40484 and 40488 in the so-called latent phase of infection presented little viral replication in the brain. These observations suggest the presence of an active immunologic control of viral replication in the CNS, though effectors of this control are unknown. Early infection of the CNS would arise as a consequence of the initial peak of viremia during primary infection and would subsequently be maintained at restricted levels by immunological control. Such a model could explain the existence of sequelae from an early episode of viral replication in the CNS and may account for the neuropathologic changes observed in latently infected animals (40484 and 40488).

Using combined immunohistochemistry and *in situ* hybridization, SIV infected cells were found to express the CD68 molecule, a marker specific of monocytes, macrophages, and microglial cells. SIV infection was localized to perivascular macrophages at the earliest stage (Figure 1G) and was found in perivascular macrophages (Figure 1H), nodule macrophages and microglial cells scattered through the brain parenchyma at later stages. T4 lymphocytes were not found to be a major source of virus in the brain as infiltrated T4 cells were few and as early infection of cells of the choroid plexus was not detected, although many of these cells stained strongly for the CD4 marker. Our results thus support the notion that infiltrating monocytes/macrophages represent the main route of entry of SIV in the CNS.

The reason why infected macrophages invade the CNS is not known. It is possible that macrophage entry represents a physiologic process as macrophages are found perivascularly in normal brain.[31] Furthermore, perivascular brain macrophages were shown to undergo a turnover from bone marrow precursors in rodents.[32] In this view, infection of the brain would parallel the systemic infection, as peripherally infected macrophages progressively migrate into the CNS. However, the occurrence of early changes, involving increased macrophage infiltration and microglia activation, suggests that an inflammatorylike process is rapidly initiated in the brain. A few initially infected cells in the brain may trigger an inflammatory reaction that would amplify as more cells, including infected ones, are recruited. Considering the limited number of infected cells present in the early brain perivascular infiltrates, an immunopathologic mechanism

could explain the enhanced recruitment of leukocytes. Induction of MHC class II expression on microglial cells also suggests a major change in the immunoregulatory environment of infected brain. Inappropriate secretion of cytokines by macrophages are believed to be involved in the generation of brain lesions in sheep infected with Visna virus.[33] Similar mechanisms may apply for primate lentiviruses and account for the activated status of macrophages and microglia throughout brain tissue.

Alternatively, astrocytes, though they are not detectably infected, could mediate an immunopathologic reaction in infected brain. Astrocytosis was one of the earliest neuropathologic changes observed and was present in two of the animals studied. Astrocytic endfeet are in close proximity to endothelial cells of the brain-blood barrier and may contribute to its integrity.[34] Furthermore, astrocytes are able to present antigens to T lymphocytes and are known to secrete cytokines.[35]Astrocytic reaction may lead to functional impairment of the brain-blood barrier and may induce endothelial changes that provoke leukocyte adhesion. How the astrocytic reaction would be triggered during the initial viremic phase remains to be determined. SIV will provide a useful model for the *in situ* study of cytokines and cell-surface protein expression in the course of lentiviral infection. This study could shed some light on the mechanisms of virus spread in the host and in the origin of lentivirus-induced immunopathology.

Acknowledgments

The authors thank R. C. Desrosiers for supplying the SIVmac251 isolate and F. Arenzana-Seisdedos for antibodies. The authors also thank M. Brahic for instigating this work, M. McChesney for helpful discussions, and G. Milon for advice and critical reading of the manuscript.

References

1. Price RW, Brew B, Sitdis J, Rosenblum M, Scheck AC, Cleary P: The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. Science 1988, 239:586–592
2. Michaels J, Sharer LR, Epstein LG: Human immunodeficiency virus type 1 (HIV-1) infection of the nervous system: a review. Immunodeficiency Rev 1988, 1:71–104
3. Elder GA, Sever JL: AIDS and neurological disorders: an overview. Ann Neurol 1988, 23(supp):S4–S6
4. Budka H, Wiley CA, Kleihues P, Artigas J, Asbury AK, Cho E-S, Cornblath DR, Dal Canto MC, DeGirolami U, Dickson D, et al.: HIV-associated disease of the nervous system: Review of nomenclature and proposal for neuropathology-based terminology. Brain Pathol 1991, 1:142–150
5. Resnick L, diMarzo-Veronese F, Schupbach J, Tourtellotte WW, Ho DD, Muller F, Shapshak P, Vogt M, Groopman JE, Markham PD, Gallo RC: Intra-blood-brain-barrier synthesis of HTLV-III specific IgG in patients with neurologic symptoms associated with AIDS or AIDS-related complex. N Engl J Med 1985, 313:1498–1504
6. Goudsmit J, Wolters EC, Bakker M, Smit L, van der Noordaa J, Hirsch EAH, Tutuarima JA, Van der Helm HJ: Intrathecal synthesis of antibodies to HTLV-III in patients without AIDS or AIDS related complex. Br Med J 1986, 292:1231–1234
7. Hollander H, Stringari S: Human immunodeficiency virus-associated meningitis. Am J Med 1987, 83:813–816
8. Ho DD, Sarngadharan MG, Resnick L, F. D-V, Rota TR, Hirsch MS: Primary human T-lymphotropic virus type III infection. Ann Intern Med 1985, 103:880–883
9. Carne CA, Tedder RS, Smith A, Sutherland S, Elkington SG, Daly HM, Preston FE, Craske J: Acute encephalopathy coincident with seroconversion for anti-LAV/HTLV-III. Lancet 1986, 2:1206–1207
10. Hollander H, Levy JA: Neurologic abnormalities and recovery of human immunodeficiency virus from cerebrospinal fluid. Ann Intern Med 1987, 106:692–695
11. Koralnik IJ, Beaumanoir A, Häusler R, Kohler A, Safran AB, Delacoux R, Vibert D, Mayer E, Burkhard P, Nahory A, Magistris MR, Sanchez J, Myers P, Paccolat F, Quoëx F, Gabriel V, Perrin L, Mermillod B, Gauthier G, Waldvogel F, Hirschel B: A controlled study of early neurologic abnormalities in men with asymptomatic human immunodeficiency virus infection. N Engl J Med 1990, 323:864–870
12. Shaw GM, Harper ME, Hahn BH, Epstein LG, Gajdusek DC, Price RW, Navia BA, Petito CK, O'Hara CJ, Groopman JE, Cho ES, Oleske JM, Wong-Staal F, Gallo RC: HTLV-III infection of brains in children and adults with AIDS encephalopathy. Science 1985, 227:177–182
13. Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA, Fauci AS: Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science 1986, 233:1089–1093
14. Vazeux R, Brousse N, Jarry A, Henin D, Marche C, Vedrenne C, Mikol J, Wolff M, Michon C, Rozenbaum W, Bureau JF, Montagnier L, Brahic M: AIDS subacute encephalitis: identification of HIV infected cells. Am J Pathol 1987, 126:403–410
15. Pang S, Koyanagi Y, Miles S, Wiley C, Vinters HV, Chen ISY: High levels of unintegrated HIV-1 DNA in brain tissue of AIDS dementia patients. Nature (London) 1990, 343:85–89
16. Weiser B, Peres N, La Neve D, Eilbot DJ, Seidman R, Burger H: Human immunodeficiency virus type 1 expression in the central nervous system correlates directly with extent of disease. Proc Natl Acad Sci USA 1990, 87:3997–4001
17. Daniel MD, Letvin NL, King NW, Kannagi M, Sehgal PK, Hunt RD, Kanki PJ, Essex M, Desrosiers RC: Isolation of a T-cell tropic HTL VIII-like retrovirus from macaques. Science 1985, 228:1201–1204
18. Chakrabarti L, Guyader M, Alizon M, Daniel MD, Desrosiers RC, Tiollais P, Sonigo P: Sequence from simian immunodeficiency virus from macaque and its relationship to other

human and simian retroviruses. Nature (London) 1987, 328:543–547
19. Francini G, Gurgo C, Guo HG, Gallo RC, Collati E, Fargnoli KA, Hall LF, Wong-Staal F, Reitz Jr. MS: Sequence of simian immunodeficiency virus and its relationship to human immunodeficiency viruses. Nature (London) 1987, 328:539–543
20. Hirsch V, Riedel N, Mullins JI: Genome organization of STLV-3 is similar to that of the AIDS virus except for a truncated transmembrane protein. Cell 1987, 49:307–319
21. Epstein LG: Lentiviral encephalitides in the immature host: a comparison of human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV) brain infection. Brain Dev 1989, 11:353–359
22. Ringler DJ, Hunt RD, Desrosiers RC, Daniel MD, Chalifoux LV, King NW: Simian immunodeficiency virus-induced meningoencephalitis: natural history and retrospective study. Ann Neurol 1988, 23(suppl):S101–S107
23. Singer RH, Lawrence JB, Rashtchian RN: Toward a rapid and sensitive *in situ* hybridization methodology using isotopic and nonisotopic probes. *In situ* hybridization: application to neurobiology. Edited by Valentino KL, Eberwine JH, Barchas JD. New York: Oxford University Press, 1987, pp. 71–96
24. Brahic M, Haase AT, Cash E: Simultaneous detection of viral RNA and antigens. Proc Natl Acad Sci USA 1984; 81:5445–5448
25. Artigas J, Niedobitek F, Grosse G, Heise W, Gosztonyi G: Spongiform encephalopathy in AIDS Dementia Complex: report of five cases. J Acquired Immune Deficiency Syndromes 1989, 2:374–381
26. Peluso R, Haase A, Stowring L, Edwards M, Ventura P: A Trojan horse mechanism for the spread of Visna virus in monocytes. Virology 1985, 147:231–236
27. Vazeux R, Cumont MC, Girard PM, Nassif X, Trotot P, Marche C, Matthiessen L, Vedrenne C, Mikol J, Henin D, Katlama C, Bolgert F, Montagnier L: Severe encephalitis resulting from coinfection with HIV and JC virus. Neurology 1990, 40:944–948
28. Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MB: Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc Natl Acad Sci USA 1986, 83:7089–7093
29. Harouse J, Wroblewska Z, Laughlin MA, Hickey WF, Schonwetter B, Gonzalez-Scarano F: Human choroid plexus cells can be latently infected with human immunodeficiency virus. Ann Neurol 1989, 25:406–411
30. Tindall B, Cooper DA: Primary HIV infection: host responses and intervention strategies. AIDS 1991, 5:1–14
31. Esiri MM, McGee JO: Monoclonal antibody to macrophages and microglial cells in human brain. J Clin Pathol 1986, 39:615–621
32. Hickey WF, Kimura H: Perivascular microglial cells of the CNS are bone-marrow derived and present antigen *in vivo*. Science 1988, 239:290–292
33. Narayan O, Clements JE: Biology and pathogenesis of lentiviruses. J Gen Virol 1989, 70:1617–1639
34. Risau W, Wolburg H: Development of the blood-brain barrier. TINS 1990, 13:174–178
35. Hertz L, McFarlin DE, Waksman BH: Astrocytes: auxiliary cells for immune responses in the central nervous system? Immunol Today 1990, 11:265–268